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Evaluation of Sub1 and Non-Sub1 Rice for Resistance to Bacterial Blight Using Submerged and Non-submerged Seedlings

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Abstract

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae;* is a major threat to rice production in Nepal. The disease is prevalent from terai to mid-hills causing variable reduction in grain yield and its quality. In terai, BB accompanied with flash floods in rainfed lowland and irrigated areas, posses frequent problems and severe crop loss in traditional and improved rice varieties. Recently, two rice varieties Swarna Sub1 and Samba Masuli Sub1 were released to mitigate the flash flood problem. A field experiment was conducted using these two Sub1 and two non-Sub1 rice varieties with their submerged and non-submerged seedlings to manage BB in a randomized complete block design during the 2013 and 2014 wet seasons at Regional Agricultural Research Station, Tarahara, Nepal. Use of submerged seedlings had a significant influence on BB disease severity and area under disease progress curve (AUDPC) values. The disease was significantly lower on plants transplanted after three to seven day submerged seedlings. The lowest BB was recorded on rice plants transplanted with seven day submerged seedlings. Disease reduction was more pronounced in Sub1 genotype when submerged seedlings were used. BB measured by AUDPC values and rice grain yield were negatively correlated with reduction of 5-6 kg ha^{-1} by one unit increase in AUDPC. Swarna Sub1 recorded the lowest BB as measured by disease severity and AUDPC values with or without use of submerged seedlings, and produced higher grain yield.

Keywords

Submergence, Bacterial Blight of Rice, Disease Resistance, Flood Tolerance

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1. Introduction

Bacterial blight (BB) of rice (*Oryza sativa* L.), caused by *Xanthomonasoryzae*pv. *oryzae*, is a major threat to rice production in Asia (Mew 1987,1989) and, in particular, Nepal (Manandhar 1987, Adhikari and Mew 1988, 1991, Adhikari et al 1999, Dangal et al 2014). The disease is widely distributed in the hills and plains of Nepal (Manandhar et al 1987, Adhikari and Shrestha 1989, Adhikari and Mew 1994). Yield reductions of up to 26%, directly attributable to bacterial blight infection, are common (Adhikari and Mew 1991), although losses may be higher

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during severe epidemics. Severe infection results in poor grain development, broken rice and deterioration in chemical and nutritional composition. Yield losses of 13-32% were reported on susceptible cultivar Taichung Native-1 (Thapa 1981).

The use of resistant cultivar is the most effective method for its management (Ou 1985, Khushet al1989) and host plant resistance has been extensively used in Nepal. So far more than 35 BB resistant (R) genes (called Xa genes) have been identified against *X. o.* pv*oryzae* (Suh et al 2013). However, over time, virulent strains of the pathogen breakdown the resistance of rice cultivars (Adhikari et al 1994b). For instance, breaking down of resistance of IRBB21 rice line harbouring dominant gene Xa-21 by some of Xoo isolates from Japan, Nepal, Korea and India have been reported in earlier studies (Lin et al 1996, Adhikari et al 1999).

Environmental factors especially temperature, humidity and rainfall directly influence development of bacterial blight in the field (Adhikari et al 1994a). In addition, cultivation of susceptible cultivars; Sona Masuli and Ranjeet, has been aggravating the disease intensity, inoculum build up and its effect on yield due to its faster spread (RARS 2014). In tropical and sub-tropical regions, higher amount of rainfall increases bacterial blight development. Sudden and heavy rainfall during the rice growing season also causes occurrence of floods in varying magnitudes and durations resulting in death of traditional rice varieties.

SUB1A gene was identified as the major determinant of submergence tolerance to mitigate the devastating wash out of rice plants due to flash floods (Xu et al 2006, Septiningsih et al 2008, Bailey-Serres et al 2010, Singh et al 2010). Using marker assisted backcrossing, the *SUB1A* gene was introgressed to eight rice varieties, including the five mega rice varieties of India and Bangladesh (Collard et al 2013). Nepal received the seeds of these varieties in 2008 and released Swarna Sub1 and Samba Mahsuri Sub1 (by the name of Samba Masuli Sub1 in Nepal) for flash flood affected areas in 2011 after testing in collaboration with International Rice Research Institute (IRRI), Philippines (Singh et al 2013). These varieties are gaining popularity among the farming communities even in flash floods non-affected irrigated and rainfed lowland areas.

The Sub1 varieties can tolerate complete submergence up to two weeks through restriction of carbohydrate consumption, chlorophyll degradation and elongation growth (Fukao et al 2006, Xu et al 2006, Fukao and Xiong 2013).Two rice chromosomal regions were reported to be associated with biotic stress and submergence tolerance based on silico data (Kottapalli et al 2006). Upon submergence, WRKY transcription factors (TFs) are induced in *Arabidopsis* and these are involved in the regulation of gene expression during biotic stress, abiotic stress, senescence, and several developmental processes (Rushton et al 2010). Many TFs mediate disease defense and abiotic stresses in tobacco (Zhang et al 2007) and rice (Liu et al 2008). Submergence activates innate immunity markers and WRKY TFs to confer higher disease resistance (Hsu et al 2013, Chaudhary et al 2015). In the present study, BB resistance was measured in two Sub1 and two non-Sub1 rice varieties bytransplanting their submerged and non-submerged seedlings during 2013 and 2014 at Regional Agricultural Research Station, Tarahara, Nepal.

2. Materials and Methods

The two-factor experiment was laid out in a randomized complete block design with three replications during the 2013 and 2014 wet seasons at Regional Agricultural Research Station, Tarahara, Nepal. Five submergence durations (0, 1, 3, 5 and 7 days) were evaluated using four rice genotypes (Swarna, Swarna Sub1, Samba Masuli and Samba Masuli Sub1).Sprouted seeds of the rice genotypes were grown in plastic trays @ 100g m^{-2}. The trays (56 cm × 36 cm × 11 cm) were filled with a mix of farm soil and farm yard manure (3:1) and fertilized with 150:22:0 N:P_2O_5:K_2O kg ha^{-1}. The trays were watered whenever necessary. Two rows of each genotype were seeded and replicated thrice within each tray. The seedlings were submerged for different periods as per assigned in a submergence tank 15 days after sowing. The trays were taken out of the tank and kept for 7 days near the tank for recovery of the seedlings.

The seedlings were transplanted in an experimental unit of two rows of two meter long spaced at 20 cm between rows and 15 cm between hills. Susceptible variety of rice (purple) was planted between plots. The fertilizer rate was 120:30:30 N:P_2O_5:K_2O kg ha^{-1}. Other agronomic practices were followed as per recommendation. The trial blocks were irrigated as and when needed. Fresh BB infected rice leaves were collected from the surrounding fields and chopped into small pieces. The chopped pieces were immersed in water for 30 minutes to prepare inoculums suspension. The suspension was inoculated on 56 days old plants using Kauffmans clipping method. The disease was scored at 14 days after inoculation and continued for three scorings at 7 days interval using standard evaluation system (0-9 scale) for rice (IRRI 1996). Scores were converted into disease severity as follows.

$$\text{Bacterial blight severity (\%)} = \frac{\text{Score recorded}}{9} \times 100$$

Area under disease progress curve (AUDPC) values were

calculated as per the procedure of Shanner and Finney (1977) using the following formula.

$$AUDPC = \sum_{i=1}^{n} \left[\frac{(y_{i+1} + y_i)}{2}\right] \times (x_{i+1} + x_i)$$

Where,

y_i = disease severity at the i^{th} observation, x_i = time at the i^{th} observation, and n = total number of observations

Disease severity data was arcsine transformed and subjected to analysis. Regression analysis between AUDPC values and grain yield was also done to understand the effect of BB on grain yield. Analysis of variance (ANOVA) was performed using MSTATC to compare the effect of submergence duration and genetic differences for bacterial blight development. The treatments were compared using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

Final BB severity was higher in 2013 than that in 2014 (Fig. 1). Submergence duration affected BB development during 2013 and 2014 wet seasons (Fig 1A). Disease severity differed significantly between plots transplanted with submerged and non-submerged rice seedlings. Plots transplanted with even one day submerged seedlings showed lower BB severity. BB development was similar on rice plants among plots transplanted with one to seven day submerged seedlings (Fig. 1A). The combined analysis of disease severity also indicated the similar result. Irrespective of rice seedlings used for transplanting after variable submergence durations, rice genotypes differed significantly for BB severity during the 2013 and 2014 wet seasons (Fig. 1B). The combined analysis also showed the similar result. Samba Masuli recorded the highest disease severity in all cases while Swarna Sub1 had the lowest disease severity.

Submergence duration affected BB development as measured by AUDPC during the 2013 and 2014 wet seasons (Table 1). During 2013, BB severity was higher as compared to BB in 2014. In 2013, BB was reduced significantly when one day submerged seedlings were transplanted. BB did not differ among the plots transplanted with one to seven day submerged seedlings. In 2014, BB varied significantly on rice plants grown after five to seven day submerged seedlings than those of plants grown without submerged seedlings. Pooled analysis also showed the significant differences for AUDPC values among plots grown with seedlings after submergence. The disease was significantly lower on plants transplanted after three to seven day submerged seedlings. The lowest BB was recorded on rice plants transplanted with seven day submerged seedlings.

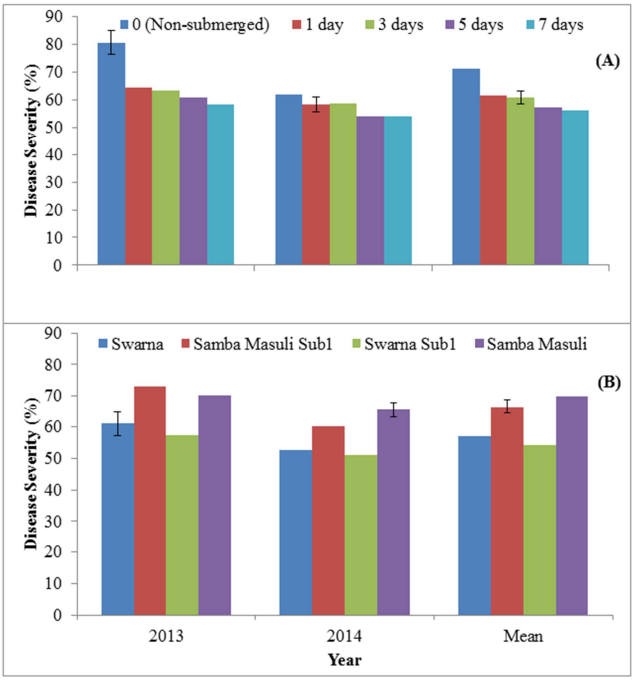

Fig. 1. Final bacterial blight disease severity (%) on four rice genotypes (Swarna, Samba Masuli Sub1, Swarna Sub1 and Samba Masuli) as influenced by five submergence durations (0, 1, 3, 5 & 7 days) during 2013 (A) and 2014 (B) at Regional Agricultural Research Station, Tarahara, Nepal.

Table 1. Effect of submergence durations on bacterial blight development in Sub1 and non-Sub1 rice genotypes as measured by area under disease progress curve (AUDPC) during the 2013 and 2014 wet seasons at Regional Agricultural Research Station, Tarahara, Sunsari, Nepal.

Submergence Duration (Days)	AUDPC		
	2013	2014	Mean
0	1057.0 a	868.5 a	962.5 A
1	998.2 b	849.1 a	923.6 AB
3	969.0 b	790.7 ab	879.9 BC
5	965.8 b	732.4 ab	849.1 C
7	939.8 b	725.9 b	832.9 C
SE	24.8	38.94	22.98
CV (%)	8.72	17.0	12.65

Table 2. Effect of rice genotypes on bacterial blight as influenced by different submergence durations and measured by area under disease progress curve (AUDPC) during the 2013 and 2014 wet seasons at Regional Agricultural Research Station, Tarahara, Sunsari, Nepal.

Rice Genotypes	AUDPC		
	2013	2014	Mean
Swarna	912.6 b	741.5 cd	827.0 B
Samba Masuli Sub1	1104.4 a	829.6 bc	967.0 A
Swarna Sub1	821.9 c	674.1 d	748.0 C
Mamba Masuli	1104.0 a	928.1 ab	1016.0 A
SE	22.2	34.83	20.55
CV (%)	8.72	17.0	12.65

Irrespective of submergence durations, rice genotypes significantly differed for BB development measured by

AUDPC during the 2013 and 2014 wet seasons (Table 2). Swarna Sub1 had the lowest BB among four genotypes. Swarna recorded the intermediate level of BB. Samba Masuli with and without Sub1 showed significantly higher BB severity in both the years.

Bacterial blight development decreased when submerged rice seedlings were transplanted (Fig. 1 and Table 1). With increased duration of submerging the rice seedlings lowered BB development in decreasing trend, with a higher reduction in Sub1 genotypes during 2013 and 2014 wet season (Fig. 2). In general, Samba Masuli recorded the highest AUDPC values under all submergence durations and Swarna Sub1 had the lowest AUDPC values under all submergence durations in the both seasons. The Sub1 genotypes can withstand complete submergence up to two weeks through restriction of carbohydrate consumption, chlorophyll degradation and elongation growth (Fukao et al 2006, Xu et al 2006, Fukao and Xiong 2013). The Sub1 gene was activated during submergence and the rice plants accumulated more carbohydrate and in turn expressed higher level of BB resistance after de-submergence. The similar results for increased resistance to stalk rot of maize were reported by Dodd (1980), when source to sink ratio was greater. Recently, Chaudhary et al (2015) reported that resistance to blast in Sub1 rice increased when submerged seedlings were used.

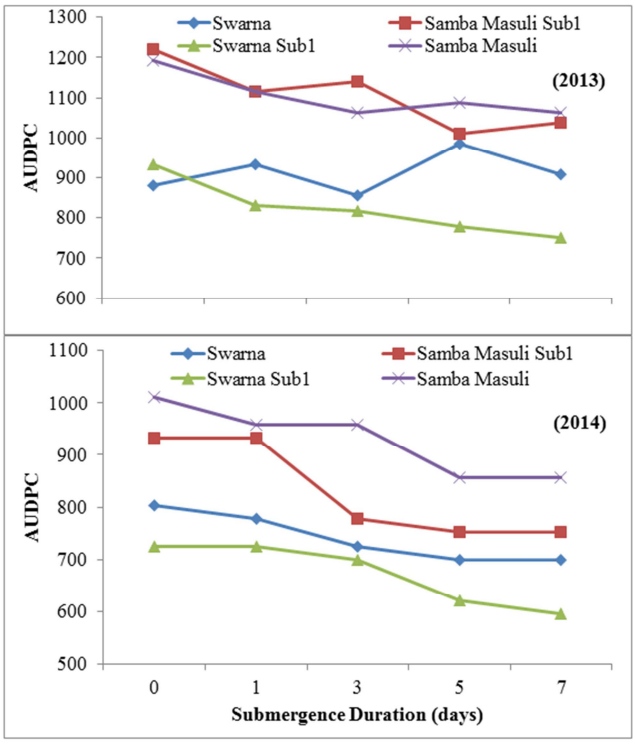

Figure 2. Pooled interaction effect of four rice genotypes (Swarna, Swarna Sub1, Samba Masuli Sub1 and Samba Masuli) and submergence durations (0, 1, 3, 5 & 7 days) over 2013 and 2014 for bacterial blight development measured by AUDPC values at RARS, Tarahara.

In general, with increasing bacterial blight AUDPC values grain yield of rice was decreasing significantly during the 2013 (R^2=-0.94) and 2014 (R^2=-0.89) wet seasons (Fig. 3). In 2013, grain yield of rice was deceased by 6 kg ha^{-1} with one unit increase in AUDPC value. Similarly, grain yield was decreased by 5 kg $ha^{-1}$with one unit increase in AUDPC value during 2014.

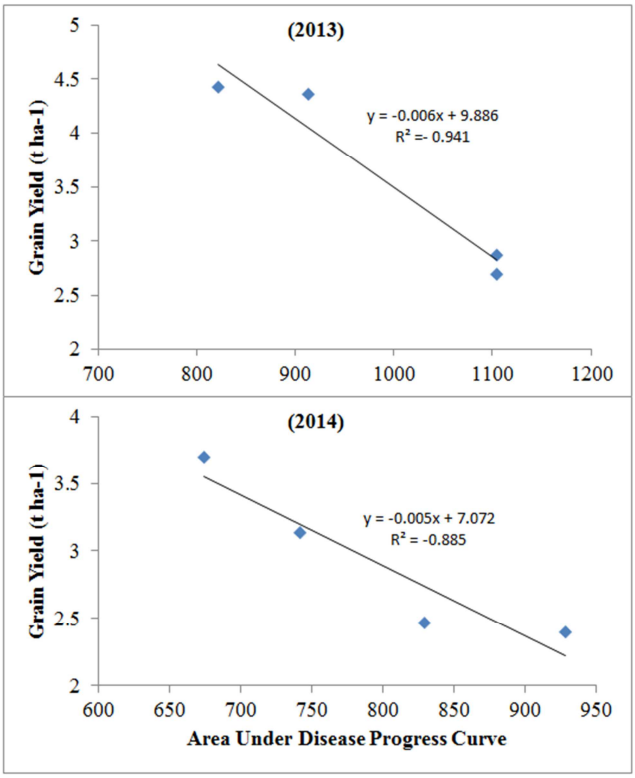

Figure 3. Relationship between area under disease progress curve of bacterial blight and grain yield of four rice genotypes as influenced by use of non-submerged seedlings and submerged seedlings for 0, 1, 3, 5 and 7 days during 2013 and 2014 wet seasons at Regional Agricultural Research Station, Tarahara, Sunsari, Nepal.

In natural conditions, flooding leads to a higher probability of pathogen infection and faster disease development in plants (Kottapalli et al 2006). However, rice plants have evolved complex sensing mechanisms and response pathways that help them adapt to diverse environmental conditions and ensure their survival (Bailey-Serres and Voeseneck 2008). Transcriptional regulation is one of the mechanisms used by plants to protect against biotic and abiotic stresses. Two rice chromosomal regions were reported to be associated with biotic stress and submergence tolerance based on in silico data (Kottapalli et al 2006). Current transcriptomic data indicate that several genes that encode WRKY transcription factor (TF) family members are induced to high levels upon flooding (Hsu et al 2011).

Rushton et al (2010) reported that WRKY TFs are induced upon submergence in *Arabidopsis* and these are involved in regulation of gene expression during biotic stress, abiotic

stress, senescence, and several developmental processes. In addition, many TFs mediate disease defense and abiotic stresses in tobacco (Zhang et al 2007) and rice (Liu et al 2008). A review also discussed about the central roles of some WRKY TFs in mediating both abiotic and biotic stresses (Friedel et al 2012). WRKY-mediated pathways are known to have a major role responsible for immune responses in plants (Eulgen and Somssich 2007). Plant innate immunity is triggered by microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) and /or effector- triggered immunity. In PAMP- triggered immunity, attack by pathogens is recognized by group of proteins termed pattern recognition receptors (RRs) that trigger a defense response (Boller and Felix 2009). Thus mitogen-activated protein kinases and calcium dependent protein kinases are activated to induce innate immunity marker genes (Asai et al 2002, Boudsocq et al 2010). These responses eventually lead to develop systemic acquired resistance (SAR) in plants, which confers immunity against subsequent infection.

Submergence activates innate immunity markers and WRKY TFs to confer higher disease resistance to plants (Hsu et al 2013). They identified a key TF, WRKY22 that mediates this response. The results not only showed the induction of WRKY genes and innate immunity marker genes in response to flooding, but also showed that pathogen resistance could be triggered by flooding. Thus the plants have evolved disease defense in response to submergence in anticipation of a higher risk of pathogen attack during- and post submergence (Hsu et al 2013).

Our studies suggested that higher bacterial resistance was observed in rice plants that had undergone submergence treatment. Among genotypes, the Sub1 genotypes expressed higher level of resistance to BB pathogen. Grain yield of rice decreased by 5-6 kg ha^{-1} with one unit increase in BB AUDPC values. Swarna Sub1 had the lowest BB with or without use of submerged seedlings for transplanting and the variety produced the highest grain yield.

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